



FINAL PROGRESS REPORT

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R&T Code 4414208

PRINCIPAL INVESTIGATOR: Dr. Edward D. Salmon  
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INSTITUTION: University of North Carolina at Chapel Hill

GRANT TITLE: Effects of Hydrostatic Pressure on Mammalian Tissue Cells - Disruption of Cytoskeletal Function, Organization, and Regulation.

REPORTING PERIOD: 1 June 1992 - 31 May 1996 (Final Report)

AWARD PERIOD: 1 April 1992 - 31 March 1996

OBJECTIVE: To investigate the disruption, organization and regulation of the cytoskeleton of mammalian tissue cells by hydrostatic pressure.

APPROACH: Novel optical and fixation chambers are being used in combination with immunofluorescence and video microscopy, biochemical and biophysical techniques to examine pressure-induced changes in the structural organization of the cytoskeletal proteins (including tubulin, actin, myosin II, vinculin, talin, vimentin, and cytokeratin) involved in producing changes in cell shape, motility and contractility.

ACCOMPLISHMENTS: (numbers in parenthesis refer to published papers)

1) We developed new microscope pressure chambers for high resolution and fluorescence studies of live cells using video microscopy (1).

2) To test if pressure alters tissue cell contractility, we have used silicone rubber substrata within our microscope optical pressure chambers and video microscopy. Mammalian fibroblasts normally exert contractile forces on their substrata which can be seen by the deformation of compliant silicone rubber. These cells undergo marked, reversible weakening of contractility beginning at moderate hydrostatic pressures (50 atm). These are novel findings and they occur at pressures which have little apparent effect on the organization and assembly of cytoskeletal proteins and cell spreading (2).

Larger pressures, in the range of 250-400 atm which disrupt actin stress fibers (2), cause complete rounding of most cells, with a total loss of contractility; this response is reversible upon pressure release. We also find that these rounded cells have not lost their adhesions to the substratum (even though they do lose adhesion site proteins (2)), but remain connected to it by long thin strands of membrane ("retraction fibers").

3) To test if pressure alters tissue cell motility, we recorded the movements of the lamellipodia at the leading edges of cells as they migrated across their substrata. Leading edge motility of fibroblasts appeared unaffected by pressure until pressures that induced cell rounding, then motility abruptly stopped.

We also investigated the lamellipod motility of fish keratocytes, which is a major cell model system used to study tissue cell motility mechanisms. Similar results were seen as for the fibroblasts. The loss of contractility seems to be at least partly responsible for the failure of keratocytes to pull away from their adhesion sites at pressures above

150 atm. Since the velocity of the lamellipod was not slowed by pressure, cells often developed long trailing processes reaching back to adhesion sites.

4) To determine the effect of pressure on the cytoskeleton and thus provide better indicators of molecular mechanisms we used fluorescent antibody staining to compare the organization of seven different cytoskeletal proteins in human HeLa cells and rat osteosarcoma cells subjected to pressures up to 400 atm. Rounded cells showed disruption of actin stress fibers and vinculin and talin at adhesion sites. Some cells remained unrounded and these showed normal distributions of these proteins. Microtubules and myosin II filaments appeared resistant to these pressures. Surprisingly, cytokeratin intermediate filaments in HeLa cells were disrupted in all cells by 200 atm. This was a surprise, because when isolated in vitro, intermediate filaments are unusually stable. Vimentin intermediate filaments in HeLa cells were sensitive to pressure while those in the osteoblasts were not. The large difference in response to pressure between different cells, the sensitivity of cytokeratin filaments and the contrast in vimentin's response in one cell line compared to the other indicate that the cellular target for pressure is not the cytoskeletal assembly reactions, but a component(s) of the regulatory mechanisms which control assembly.

5) To test if pressure affected cytoskeletal organization through a calcium regulatory pathway, we used the fluorescent dye Fura-2 to determine changes in cytosol  $\text{Ca}^{2+}$  concentrations (1). This study required accurate calibration of how pressure effects Fura-2 fluorescence. We found no changes in  $\text{Ca}^{2+}$  concentrations at 400 atm.

6) Thus, we have now re-directed our efforts to discover what phosphorylation pathways within cells are the targets for pressure's disruption of the cytoskeleton. This will require new methods for measuring specific kinase and phosphatase activities under pressure.

SIGNIFICANCE: The molecular mechanisms of the effects of high hydrostatic pressure on living mammalian cells are poorly understood, but appear to involve the cytoskeleton, since pressure disrupts tissue integrity, inhibits cell proliferation, blocks contractility and may disrupt intracellular transports. These effects limit human diving in the deep sea and may complicate hyperbaric therapies.

#### PUBLICATIONS AND MANUSCRIPTS IN PREPARATION

1. Crenshaw, H. C. and E. D. Salmon (1996) Hydrostatic pressure to 400 atm does not induce changes in the cytosolic concentration of  $\text{Ca}^{2+}$  in mouse fibroblasts: measurements using Fura-2 fluorescence. Exp. Cell Res. 227: 277-284.

2. Crenshaw, H. C., J. A. Allen, V. Skeen, A. Harris, and E. D. Salmon (1996) Hydrostatic pressure has different effects on the assembly of tubulin, actin, myosin II, vinculin, talin, vimentin, and cytokeratin in mammalian tissue cells. Exp. Cell Res. 227: 285-297.

3. Harris, A, J. Allen, H. C. Crenshaw and E. D. Salmon (In preparation) Hydrostatic pressure alters the contractility by not lamellipod motility of fibroblasts and fish keratocytes.

POTENTIAL PATENTABLE INVENTIONS: NONE

ANNUAL REPORT QUESTIONNAIRE (195-96)  
(for ONR use only)

Principal Investigator: E.D. Salmon and A.K. Harris  
Institution: UNC - Chapel Hill  
Project Title: Hydrostatic Pressure Disruption of Mammalian Tissue  
Cell Cytoskeletal Function, Organization and Regulation

Number of ONR supported

Papers published in refereed journals: 2  
Papers or reports in non-refereed publications: 0  
Books or book chapters published: 0

Number of ONR supported patents/inventions

Filed: 0

Granted: 0

Patent name(s) and number(s): \_\_\_\_\_

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Number of presentations: Total ONR Project

Invited: 0

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Trainee Data (only for those receiving full or partial ONR  
support):

TOTAL FEMALE MINORITY NON-US CITIZEN

No. Grad. Students:

No. Postdoctorals: 0

No. Undergraduates: 2

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AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP  
(please describe):

E. D. Salmon - Lyle V. Jones Professorship

Equipment purchased on grant (number and description of items  
costing >\$1,500):